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Original Article

BIOANALYTICAL OF UPLC METHOD DEVELOPMENT AND VALIDATION OF XANTHORRIZOL AND ITS APPLICATION TO PHARMACOKINETIC STUDY

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ABSTRACT

Objective: A simple, straightforward, ultra-performance liquid chromatography (UPLC) with a fluorescence detector method was developed and validated to determine xanthorrhizol in rat plasma. This method was successfully applied to an oral pharmacokinetic study.

Methods: Xanthorrhizol was separated using a C18 column in an isocratic mode using a mobile phase of acetonitrile: water (85:15 v/v) at a 0.4 ml/min flow rate. The fluorescence detector was set at 230 nm excitation and 320 nm emission wavelengths. The method was then applied in the pharmacokinetic study involving 12 Sprague-Dawley rats.

Results: The developed bioanalytical methods were found to be linear in the range of 0.078–5 µg/ml with a correlation coefficient of r^2=0.999. The percentage recovery of xanthorrhizol was more than 95%, and the relative standard deviation was less than 2. These results indicate that the method is accurate and precise. The limit of detection (LOD) and limit of quantification (LOQ) of the technique were 0.123 µg/ml and 0.373 µg/ml, respectively. Furthermore, the stability studies demonstrated that xanthorrhizol is stable under various analytical conditions. The pharmacokinetic study revealed that the area under the curve (AUC) was 27.23±19.65 (µg. h/ml), the half-life ($t_{1/2}$) was 7.71±2.89 h, the mean residence time (MRT) was 13.86±4.06 h while the maximum concentration (C_{max}) was 1.58±0.62 µg/ml, and the time to reach the maximum concentration (T_{max}) was 1.33±0.20 h.

Conclusion: The developed bioanalytical method was reliable and successfully met all validation criteria, making it a robust choice for quantifying xanthorrhizol. Therefore, it may be effectively utilized to determine xanthorrhizol in rat plasma following a pharmacokinetic study.

Keywords: Xanthorrhizol, UPLC, Method validation, Rat plasma, Pharmacokinetic study

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INTRODUCTION

Xanthorrhizol is a bisabolene sesquiterpenoid compound initially isolated from the rhizome of *Curcuma xanthorrhiza* Roxb [1] together with curcumin [2]. *Curcuma xanthorrhiza,* known as java turmeric or temulawak, is from the *Zingiberaceae* family. This plant is commonly used as a component of the traditional herbal remedies jamu (Bahasa) or used independently to cure medical problems such as indigestion, rheumatism [3, 4], kidney stones, fever, and high cholesterol levels [5, 6]. Xanthorrhizol has an IUPAC name (5-(1,5-dimethyl-4-hexenyl)-2-methylphenol [7] with a chemical structure shown in fig. 1.

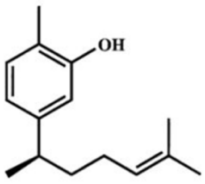

Fig. 1: Chemical structure of xanthorrhizol [1]

Previous studies demonstrated that xanthorrhizol has various pharmacological activities such as antibacterial activity against dental caries-causing bacteria [8, 9] and endodontic infection [10], antiacne-causing bacteria (*Propionibacterium acnes)* [5], anticandidal activity [11, 12], antifungal activity against planktonic fungal cells such as *Malassezia* species [13], prevent dental plaque [14], anti-inflammatory [15-19], antioxidant [16, 20], anticancer [21], and antiplatelet [22]. Moreover, xanthorrhizol exhibited hepatoprotective and nephroprotective activity by reducing the specific gravity of the kidney caused by cisplatin [23]. Meanwhile, for antiaging, xanthorrhizol induced the expression of matrix metalloproteinase 1 (MMP-1) and increased the expression of type-1 procollagen in ultraviolet-irritated human skin fibroblast [24].

Considering the therapeutic potential of xanthorrhizol in various pharmacological activities, it is essential to conduct pharmacokinetic studies to understand its safety and efficacy in the body [25]. Thus, it is worth developing a simple, precise, and accurate analytical method to quantify the drug molecule in various biological fluids, particularly plasma. Several researchers have reported the pharmacokinetics profile of xanthorrhizol [26, 27]. For instance, Choi *et al.* conducted an oral pharmacokinetic study to compare the bioavailability of pure xanthorrhizol and java turmeric extract. The study found that the absolute oral bioavailability of pure xanthorrhizol and java turmeric extract (containing 30% xanthorrhizol) was 12.9% and 13.4%, respectively. This data could be used to select the appropriate maintenance dose of xanthorrhizol and java turmeric extract and convert an intravenous dose to an oral dose [27].

A few analytical methods, such as gas chromatography (GC) with mass spectrophotometry (MS) method [28] and liquid chromatography method with UV-Vis detection [29, 30], have been published for the quantification of xanthorrhizol in crude extract from several plants. However, only Choi *et al.* reported a validated analytical method based on high-pressure liquid chromatography in tandem with mass spectrometry (MS/MS) to determine xanthorrhizol in biological samples. In this method, the plasma sample was treated with a deproteinizing solution to precipitate the protein, thereby releasing the compound [26]. Despite the fact that liquid chromatographic methods utilizing MS/MS systems have been used for the analysis of the curcumin [31], delafloxacin [32], oleandrin, and adynerin [33], these methods require operation by professionally trained personnel and sophisticated equipment [34].

On the other hand, there have been no validated analytical methods for the determination of xanthorrhizol in biological samples using ultra-performance liquid chromatography (UPLC) with a fluorescence detector, even though this method has been widely used for the bioanalytical of several compounds, such as S-adenosylmethionine [35], doxorubicin, and prodigiosin [36], tetrahydro palmitate and cocaine [37]. Fluorescence has a higher specificity and lower background than other detection methods, resulting in a higher signal-to-noise ratio [38].

This study aimed to develop a simple, rapid, and sensitive ultra-performance liquid chromatography (UPLC) with a fluorescence detector method for determining xanthorrhizol in rat plasma. The method was optimized and validated without the need for an internal standard. Simple sample preparation (e. g., protein precipitation) with high recovery of xanthorrhizol was used. The method was validated according to USFDA guidelines, including accuracy, precision, specificity, and drug stability in plasma. Various parameters were evaluated to ensure the adequate validation of the method. The method was then implemented to measure xanthorrhizol concentration in rat plasma following a pharmacokinetic study.

MATERIALS AND METHODS

Materials and reagents

Xanthorrhizol was purchased from Javaplant (Jakarta, Indonesia) and used as a model compound. HPLC-grade acetonitrile, tetrahydrofuran, and methanol were supplied from Merck (Darmstadt, Germany). Ultrapure water (18.2 MΩ cm at 25 °C) was obtained from Reservoir® Elga Water System (High Wycombe, UK).

Animal and blank rat plasma

The animal experiments were conducted following the guidelines of the Committee on Animal Research and Ethics (CARE) of the Faculty of Pharmacy, UiTM. (UiTM CARE: 416/2023). The blood samples were withdrawn from healthy Sprague-Dawley (SD) rats weighing (200-250g). The blood samples were placed in BD Vacutainer® blood collection tubes (Franklin Lake, USA) equipped with equipped with 75 USP units of lithium heparin, thus preventing coagulation. Then, blood samples were centrifuged at 5000 rpm for 15 min using a microcentrifuge (Eppendorf 5424, Germany). The collected blank rat plasma was stored at-20 °C before use [39].

Instrumentation and chromatographic condition for the analytical method

A modern ultra-performance liquid chromatography (UPLC) system (Waters Corp., Milford, MA, USA) comprises a Water Binary Solvent Manager pump, Sample Manager, and fluorescent detector. The chromatographic separation was performed on a Gemini® C18 column (3 µm, 150 x 4.6 mm) (Phenomenex), which was fitted with a VanGuard Pre-Column packed with nova-Pak cartridge (Phenomenex). The mobile phase consisted of a mixture of acetonitrile and water (85:15, v/v) and was pumped through the system at a flow rate of 0.4 ml/min at a pressure of 12.000-13.000 psi. The wavelength detection was set to 230 nm excitation and 320 nm emission, and the sensitivity was adjusted to 0.0001 absorbance units full scale (AUFS). A 0.45 µm filter paper (Millipore) was used to filter all the solvents. The samples were quantified based on the area under the curve peak by isocratic mode, and the data acquisition run time was 12 min.

Preparation of stock and working solution

The stock solution of xanthorrhizol was prepared by accurately weighing the compound and diluting it in methanol to achieve a final concentration of 1 mg/ml [40]. The stock solution was stored in a refrigerator at 4 °C and used within a month from the date of preparation. The working solutions were obtained by serially diluting the stock solution with methanol. The concentration of the working solutions was 5, 2.5, 1.25, 0.625, 0.312, 0.156, and 0.078 µg/ml, respectively.

Preparation of calibration standard

The calibration standard was constructed by spiking plasma with a known concentration of xanthorrhizol ranging from 0.078–5 µg/ml. The calibration standard solution was stored in amber bottles at-20 °C. The calibration standard was also used to determine the within-day and between-day precision and accuracy (n=6) of the methods.

Sample preparation

To prepare the sample, 100 µl of spiked rat plasma was measured into an Eppendorf microcentrifuge tube (polypropylene; 1.5ml). The sample was then de-proteinized by adding 200 µl of a mixture of acetonitrile-tetrahydrofuran (7:3, v/v). The mixture was vortexed vigorously for 3 min using a vortex mixer (IKA, Germany) and then centrifuged at 12,800x g for 20 min in an Eppendorf 5424 microcentrifuge (Sarstedt, Germany) [41]. Subsequently, a 50 µl aliquot of the supernatant was injected into the UPLC.

Validation of UPLC method

The methods were validated according to The United States Food and Drug Administration (USFDA) guideline [42].

Selectivity

The selectivity of an analytical method is its ability to accurately distinguish and measure multiple analytes in the presence of known interferences, such as synthetic precursors, excipients, enantiomers, and known degradants. The selectivity and specificity of the method should be evaluated by processing blank samples with and without the addition of analytes and then analyzing them for the presence of interferences. The chromatogram of the rat plasma spiked with xanthorrhizol was compared with the blank plasma sample to assess the selectivity of the method.

Linearity

The linearity of the method was determined in triplicate for seven dilutions of the standard solution. The rat plasma was spiked with xanthorrhizol standard solution to give final concentrations of 0.078, 0.150, 0.31, 0.62, 1.25, 2.5, and 5 µg/ml. The calibration curve was constructed by plotting the peak area of xanthorrhizol versus the xanthorrhizol concentration. The linearity of the method was indicated by the least square regression analysis, which produced a straight line through the data points.

Accuracy

The accuracy of the analytical method was determined within the day and between days by spiking rat plasma with xanthorrhizol standard solutions (n = 6). Six replicates of each concentration were processed on the same day for intraday accuracy. For intraday accuracy, a single sample of each concentration was analyzed daily over six days, with a calibration curve constructed on each analysis day. Accuracy was expressed as a percentage of the absolute recovery.

$$\text{Absolute recovery (\%)} = \frac{\text{Actual concentration recovery}}{\text{Theoritical concentration}} \text{ x } 100$$

Precision

The repeatability and intermediate precision of the analytical method were determined by spiking rat plasma with xanthorrhizol standard solutions (n = 6). Six replicates of each concentration were analyzed on the same day for repeatability study. For the intermediate precision, a single sample of each concentration was analyzed daily over six days, with a calibration curve constructed on each day of analysis. The precision of the system was expressed as the relative standard deviation (RSD, %).

Determination of limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) was calculated based on the standard deviation of the response and the slope obtained from the linearity plot. The limit of detection was calculated as follows:

$$LOD = 3.3\alpha/S$$

Where α is the standard deviation of the y-intercept of the calibration curve, and S is the slope of the regression line.

The limit of quantification (LOQ) was calculated based on the standard deviation of the response and the slope obtained from the linearity plot. The limit of quantification was calculated as follows:

$$LOQ = 3.3\alpha/S$$

Where α is the standard deviation of the y-intercept of the calibration curve, and S is the slope of the regression line.

Recovery

The recovery of the liquid-liquid extraction (n=6) was determined by comparing the amount of analyte extracted from the sample to the amount of analyte in the unextracted standard. The absolute recovery of the extraction method was calculated as described by the equation below:

$$\text{Recovery (\%)} = \frac{\text{Peak area of xanthorrhizol in plasma}}{\text{Peak area of xanthorrhizol in methanol}} \text{ x } 100\%$$

The recovery of xanthorrhizol was determined at three quality control (QC) concentrations: 5 µg/ml (high), 1.2 µg/ml (medium), and 0.156 µg/ml (low). Six replicates were analyzed for each concentration. The mean recovery for each concentration was calculated and should be within 85-115% of its average concentration [43].

Stability

The stability of xanthorrhizol in rat plasma was evaluated by analyzing three samples (n = 3) containing high, medium, and low concentrations. The percentage concentration deviation was calculated after comparing the results with freshly prepared samples. The stability study was conducted according to the USFDA-recommended criteria. The protocol was as follows: (i) Freeze-thaw stability, as measured by three consecutive freeze-thaw cycles. The protocol was as follows: (i) Freeze-thaw stability, as measured by three consecutive freeze-thaw cycles (freezing temperature −20℃ for 24 h and thawing at room temperature). (iii) Long-term stability of samples was determined after keeping the samples frozen at −20 ℃ for 30 d. (iv) Post-preparative/autosampler stability studies were determined after keeping the sample in autosampler at 4 ℃ for 24 h. (v) The working solution stability was determined by exposing the working solution at room temperature for six hours.

Pharmacokinetic study

All animal studies performed were approved by the Committee on Animal Research and Ethics guidelines at the Faculty of Pharmacy, Universiti Teknologi MARA (UiTM CARE: 416/2023). Sprague Dawley rats (n=12) with a mean body weight range of 300–350 g were fasted for 12 h prior to the experiments but had free access to water. A single dose (25 mg/kg body weight) of xanthorrhizol was administrated orally to the rats. The blood was collected and withdrawn from the lateral caudal vein of the rat tail at pre-determined time intervals of 0, 1, 2, 3, 4, 6, 8, 10, and 12 h. The plasma sample was treated using a sample preparation method and then subjected to UPLC analysis to quantify the drug concentration. The pharmacokinetic parameters including area under the curve (AUC), MRT, $t_{1/2}$, $C_{max,}$ and T_{max} of oral absorption were calculated using Kinetica Version 5.0 software (Thermo Fisher Scientific, Philadelphia, USA).

RESULTS AND DISCUSSION

Various variables might affect the efficiency of UPLC methods regarding separation and resolution, such as the type of stationary phase, detector, detection wavelength, and chemical composition of the mobile phase [44]. Therefore, a bioanalytical approach was developed to optimize the chromatographic condition to separate xanthorrhizol in rat plasma. A fluorescence detector was selected as a detector as it is frequently used in UPLC analysis due to the ability to discriminate an analyte from interferences or background peaks [38].

The separation of xanthorrhizol in rat plasma samples was achieved by isocratic elution at a wavelength detection set of 230 nm excitation and 320 nm emission. The excitation wavelength was selected based on the UV absorption spectrum of xanthorrhizol, which indicates the energy required to excite an electron to a higher quantum state. The emission wavelength was scanned while the excitation remained constant at a defined wavelength.

The mobile phase composition, concentration, and other factors were optimized to enhance the resolution and accuracy of the chromatograms. The separation of plasma interference from xanthorrhizol was examined using various ratios of the mobile phase, water, and acetonitrile. The drug compound was successfully isolated from the plasma matrix, and a sharp peak was obtained using a mobile phase of acetonitrile: water (85:15 v/v) without the addition of any buffer. The summary of the chromatographic condition is described in table 1.

Table 1: Summary of chromatographic conditions

Parameter	Description
Equipment	Ultra-performance liquid chromatography (Waters Corp., Milford, MA, USA) comprises a Water Binary Solvent Manager pump, Sample Manager, and fluorescent detector
Column	Gemini® C18 column (3 µm, 150 x 4.6 mm)
Mobile phase	Acetonitrile and water (85:15, v/v)
Flow rate	0.4 ml/min
Column and sample temperature	Ambient 25 °C
Detection wavelength	230 nm excitation and 320 nm emission
Injection volume	10 µl
Run time	12 min
Retention time	9.05 min

Selectivity and specificity

Specificity is the ability to separate the analyte from other components in a biological fluid, such as the matrix [44]. This is done by comparing the chromatogram of the plasma spiked with the analyte to the chromatogram of blank plasma. The method is considered selective and specific if there is no interference between endogenous component peaks and active compound peaks [45].

The retention time of xanthorrhizol in rat plasma was found to be 9.05 min. As shown in fig. 2, the blank plasma chromatogram does not have a peak at this retention time, indicating that the plasma matrix did not interfere with the analysis of xanthorrhizol. The blank plasma was effectively separated from the peaks of xanthorrhizol. Therefore, the method is considered specific and selective for the determination of xanthorrhizol in rat plasma.

Linearity

The linearity range in the quantification method establishes a direct correlation between test performance and drug content in a defined range in the biological sample [44]. A 7-point calibration curve was constructed for xanthorrhizol in rat plasma (fig. 3) using concentrations that were selected based on the probability of the drug level during pharmacokinetic studies. The calibration curve showed excellent linearity over the range of 0.078 to 5 µg/ml. A 1/x weighted method was used to fit the calibration curve to a linear equation, Y=341119.4+3560844X, with a regression coefficient (r^2) of 0.999, indicating a good fit of the data to the linear model.

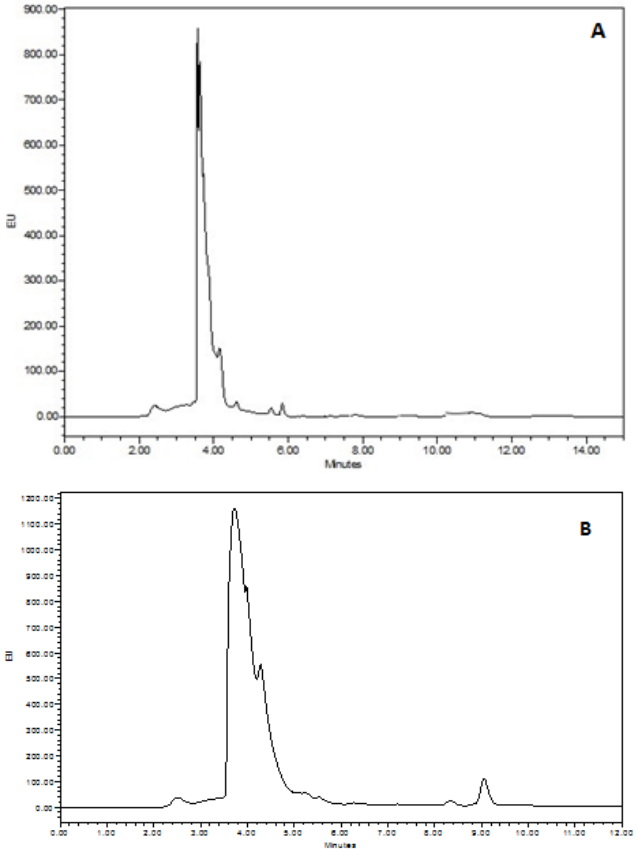

Fig. 2: Chromatogram of A) rat blank plasma, B) rat blank plasma spiked with 5 µg/ml of xanthorrhizol

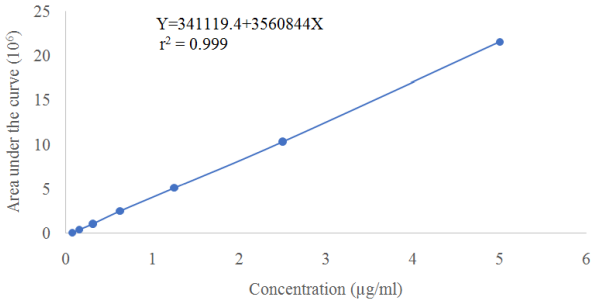

Fig. 3: Calibration curve of xanthorrhizol

Accuracy and precision

The accuracy determines the closeness of the measurement to the true value. At the same time, precision refers to the closeness of repeated measurements to each other [44]. According to USFDA guidelines for bioanalytical methods validation, the mean value should be within 15% of the nominal value. Accuracy and precision were expressed as a percentage of absolute recovery and RSD. The within-day and between-day accuracy and precision of the method are summarized in table 2. The accuracy of the method for within-day and between-day varied between 95.47% to 103.19% and 95.17 to 99.79%, respectively. At the same time, the precision value of the method ranged from 1.080 to 1.94 % for within-day and 0.23 to 1.94% for between-day. These results indicate that the method has adequate precision and accuracy and is reliable for the determination of xanthorrhizol in rat plasma samples.

Table 2: Within-day and between-day precision and accuracy of xanthorrhizol

Theoretical concentration (ng/ml)	Accuracy (%)		Precision (RSD %)	
	Within-day	Between-day	Within-day	Between-day
5000	103.198	98.243	1.942	1.604
2500	98.775	95.816	1.654	0.717
1200	99.670	96.816	1.080	1.829
625	101.360	99.794	1.594	1.944
312	96.729	96.950	1.275	1.156
156	99.041	96.321	1.388	1.097
78.125	95.477	95.175	1.632	0.234

*(n=6 at each concentration)

Determination of limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) of xanthorrhizol were found to be 0.123 µg/ml and 0.373 µg/ml, respectively.

Recovery

Xanthorrhizol is a lipophilic compound that is bound to lipoproteins in plasma. Liquid-liquid extraction is a simple and cost-effective method for extracting xanthorrhizol from plasma samples. Thus, the recovery study was performed to investigate the effectiveness of the extraction procedure of xanthorrhizol in plasma.

The liquid-liquid extraction method is more cost-effective than the solid-liquid extraction [46]. This method uses a deproteinization solution to precipitate proteins from the plasma sample, thereby releasing xanthorrhizol. The polarity of the deproteinized solution and the resulting mixture must be optimized to dissolve the release of xanthorrhizol. In this study, we investigated the optimization of the use of deproteinized agents such as methanol, ethanol, acetonitrile, propanol, and tetrahydrofuran (THF). Fig. 4 shows that acetonitrile and propanol have the highest percentage of recovery, 71.884%, and 78.060%, respectively. Meanwhile, ethanol, methanol, and THF have similar recovery rates in the range of 60.191-62.063%. Although acetonitrile is a better deproteinizing agent than propanol, it cannot completely extract xanthorrhizol from plasma samples. To improve the extraction efficiency of acetonitrile, it was combined with THF at different ratios. THF is a polar solvent that plays a crucial role in the solubility of lipophilic compounds [47].

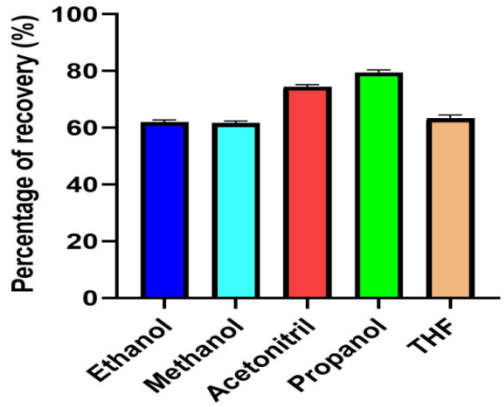

Fig. 4: The Absolute recovery of xanthorrhizol in various deproteinizing solutions. Results are expressed as mean±SEM of 3 experiments (n=3)

In addition, acetonitrile is combined with THF at various ratios to increase the extraction efficiency of xanthorrhizol from plasma samples. Previous research has shown that a combination of acetonitrile and THF can increase the absolute recovery of tocotrienol and tocopherol in rat plasma [41]. Since xanthorrhizol is also a lipophilic compound, it is possible that THF can work just as effectively to increase the solubility of xanthorrhizol.

Fig. 5 shows that a combination of acetonitrile and THF with a ratio of 7:3 achieved the highest percentage of recovery compared to other ratios. These findings suggest that THF is essential for enhancing the solubility of xanthorrhizol following its release from plasma during the deproteinization process. Therefore, a 7:3 mixture of acetonitrile and THF is the optimal deproteinization solution, as it provides the optimal polarity of the solution mixture to effectively extract xanthorrhizol from the plasma sample.

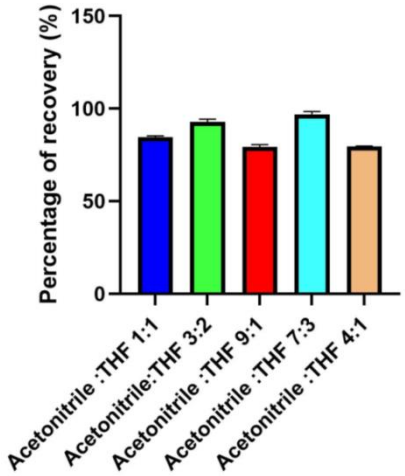

Fig. 5: The Absolute recovery of the combination of acetonitrile and THF. Results are expressed as mean ± SEM of 3 experiments (n=3).

The absolute recovery of xanthorrhizol from rat plasma using a combination of acetonitrile and THF in a 7:3 ratio as a deproteinizing agent is shown in table 3. The study was performed at a three-level quality control concentration of xanthorrhizol: 5 µg/ml (high), 1.2 µg/ml (medium), and 0.156 µg/ml (low). The recovery ranged from 99.341-103.198%, which is considered adequate and acceptable. An internal standard was not employed because this method only required the direct injection of plasma samples after a short deproteinization process.

Table 3: The absolute recovery of xanthorrhizol treated with an acetonitrile-tetrahydrofuran (7:3) deproteinizing solution

Xanthorrhizol concentration (µg/ml)	% Recovery	
	Mean (%)	RSD (%)
5	103.198	1.942
1.2	99.670	1.080
0.15	99.341	1.615

*(n = 3 at each concentration)

Stability

The stability study was conducted for three quality control sample concentrations, as shown in table 4. The results reveal that the percentage of recovery was more than 95%, and RSD was less than 2%. This indicates that no xanthorrhizol deterioration was detected under all tested conditions. Furthermore, the sample that had undergone three freezing and thawing cycles demonstrated that stability was unaffected.. According to short-and long-term stability, the sample could be stored without influencing accuracy and precision. The results from post-preparative and working solution stability tests imply that the samples remain stable throughout the sample preparation and analysis process.

***In vivo* pharmacokinetic study utilizing the validated method**

The bioanalytical method was applied to the pharmacokinetic study of xanthorrhizol in healthy male Sprague Dawley rats following oral administration. The results, depicted in Fig. 5, show the xanthorrhizol concentration in plasma over time.

Table 4: Stability data of xanthorrhizol in rat plasma for three quality control samples

Stability	Nominal concentration (ng/ml)	Recovery (%)	RSD (%)
Freeze-thaw stability (three cycles, −20°C)	5000	95.295	0.248
	1200	97.166	0.171
	156	98.495	1.919
Short-term stability (24 h, room temperature)	5000	99.414	0.149
	1200	99.514	0.167
	156	99.098	1.034
Long-term stability (30 d, −20°C)	5000	95.108	0.145
	1200	95.100	0.185
	156	97.289	0.919
Post-preparative stability (24 h, 4°C)	5000	99.452	0.205
	1200	99.514	0.167
	156	99.701	1.110
Working solution stability (8 h, room temperature)	5000	99.826	0.389
	1200	99.044	1.478
	156	99.762	1.114

*(n = 3 for each experiment)

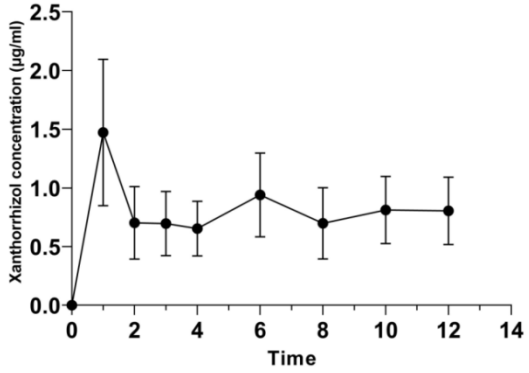

Fig. 6: The mean plasma concentration of xanthorrhizol versus time profile after oral administration in rats (n=12). Results are expressed as mean ± SEM of 12 rats.

The validated method was successfully used to determine the amount of xanthorrizol in rat plasma at pre-determined time intervals from 0 to 12 h after oral administration. The summary of pharmacokinetic parameters is described in table 5.

Table 5: The pharmacokinetic parameters, AUC, C_{max}, T_{max}, half-life (T½), and mean residence time (MRT) following dose oral administration

Parameter	Xanthorrhizol
T½ (h)	7.76±2.89
MRT (h)	13.86±4.06
Dose administered (mg/kg)	25
AUC (µg. h/ml)	27.23±19.65
C_{max} (µg/ml)	1.58±0.62
T_{max} (h)	1.33±0.20

*Data are expressed as mean±SEM (n=12). MRT-The mean residence time; AUC-Area under the curve; C_{max}-The maximum plasma concentration; Tmax-The time to reach peak concentration; T½-The half-life

Based on the pharmacokinetic parameters, we found that xanthorrhizol was absorbed in the gastrointestinal tract and reached its maximum concentration in the blood (C_{max}) of 1.58 µg/ml at 1.33 h. The half-life $T^{1/2}$ of xanthorrhizol was 7.76 h, and the drug remained in the blood circulation for a total of 13.86 h. These findings are supported by a previous study conducted by Choi *et al.*, who reported that $t_{1/2}$ of xanthorrhizol was determined at 7.5 h following oral administration of a java turmeric extract using HPLC-MS/MS detectors [27]. However, the other pharmacokinetic parameters of xanthorrhizol reported in the study differed from those in the Previous work. The previous study reported a C_{max} of 18 ng/ml, a T_{max} of 0.4 h, and an AUC of 93 ng. h/ml. The values obtained in our study were comparatively higher than those reported in the current work (C_{max} = 1.58 µg/ml, T_{max} = 1.33 h, and AUC = 13.86 µg. h/ml). The observed variations in the pharmacokinetic parameters can be attributed to several reasons, including variations in the methodologies employed for drug level determination, differences in the populations under study, or variations in the drug dosage administered [25].

CONCLUSION

A precise, simple, and sensitive liquid chromatography with a fluorescence detector method was developed and validated for the determination of xanthorrhizol in rat plasma. The method was based on a simple protein precipitation extraction procedure without the need for internal standards. In addition, all validation parameters were within the limits specified by the United States Food and Drug Administration (USFDA) guideline.

ABBREVIATION

UPLC-Ultra Performance Liquid Chromatography, LOD-Limit of Detection, LOQ-Limit of Quantification, AUC-Area Under the Curve, MRT-Mean Residence Time, C_{max}-Maximum Plasma Concentration, T_{max}-Time to Reach the Maximum Concentration, $t_{½}$-The half-life, MS-Mass Spectrophotometry, UV/Vis-Ultraviolet/Visible, USFDA-United States Food and Drug Administration, HPLC-High-Performance Liquid Chromatography, SD-Sprague Dawley, RSD-Relative Standard Deviation, THF-Tetrahydrofuran

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CONFLICT OF INTERESTS

Declared none

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